THE SUSCEPTIBILITY OF THE ALPHA–1 AND ALPHA–2 COMPONENTS OF COLLAGEN TO PEPSIN

by

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Abstract: The α1- and α2-components of denatured rat-tail-tendon collagen differ in their susceptibility to pepsin. The α2-component is first broken down to a mixture which gives the characteristic gel-electrophoretic pattern.

In previous papers we have demonstrated the starch-gel electrophoretic pattern of the pepsin-digested denatured rat-tail-tendon collagen (7) and described the fractionation of the digest (6) and the characterization (9) of two fragments. Because the amino acid compositions differ between the various peptide chains (8), it seemed justified to undertake analogous experiments with isolated collagen components.

MATERIAL AND METHODS

Collagen. — The preparation of acetic acid-soluble collagen from rat-tail-tendons has been described earlier (6, 7). The lyophilized material was stored at 4°C in a vacuum exsiccator and for further elaboration dissolved into 0.5 % acetic acid to a solution containing about 0.5 % (w/v) of protein.

Preparation of the components. — For the preparation of the α2-, α1-, β_{11}- and β_{12}-components the first attempts were based on the isolated αα- and ββ-fractions obtained by preparative gel electrophoresis (3), which were further divided in to the components mentioned with a CM-cellulose column using a linear gradient (8). The α1- and α2-components resolved well (Fig. 1), but the yields were poor and the manipulations tedious.

Later denatured collagen was divided initially into ($\alpha1+\beta_{11}$)- and ($\alpha2+\beta_{12}$)-fractions with the CM-cellulose chromatography mentioned (Fig. 2). The proteins in the eluents were recorded with a Hitachi-Perkin Elmer Model 139 spectrophotometer. Altogether 16 runs were made. The fractions were pooled, a drop of octanol added and the samples dialyzed against 0.5 % acetic acid for one week at 4°C. The final purification was performed with the preparative starch-gel electrophoresis (3). The resulting solutions were lyophilized, the residues dissolved into 0.5 % acetic acid at 40°C, dialyzed in the cold against acetate buffer (pH 4.7, $\mu=0.17$) for 48 hr and centrifuged at 17 000 *g* for 30 min. The larger part of the disturbing starch remained in the pellet. The products were rather satisfactory in the analytical starch-gel electrophoresis. The α2-component was slightly degraded to bands of faster migration rate and the α1-component contained some β_{11}-components. The α2-components were very susceptible to thermal degradation and the β_{12}-components rapidly broke down to α2- and α1-components. On the other hand, α1 was rather resistant and had a tendency to form larger aggregates resembling β_{11}. The localization of the α3-component remained obscure in the present experiments, but it may be included in the α1-component.

Pepsin treatment. As a rule the incubation with pepsin (7) lasted 3—6 hrs at 25°C with an enzyme: substrate ratio of 1:50 or 1:100. A control sample was treated similarly but without pepsin. In certain experiments more pepsin and a higher temperature was used.

RESULTS AND DISCUSSION

The conclusion from numerous experiments was that it is very difficult to degrade the isolated α1-component to clear subunits with pepsin. Even at the enzyme: substrate ratio of 1:20 or at 40°C for 24 hrs there was no clear degradation of the α1-component. Only weak bands appeared, which migrated just before the α1-band

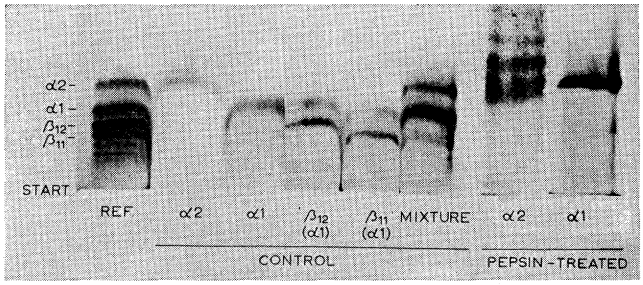

Fig. 1. — Starch-gel electrophoretic patterns of pepsin-treated purified $\alpha 1$- and $\alpha 2$-components. The digestion of the $\alpha 1$-component was carried out at 40°C in a HCl-acetic acid buffer, pH 1.0—1.5 for 6 hr, enzyme: substrate=1:100. The digestion of the $\alpha 2$-component was carried out at 25°C in a HCl-acetic acid buffer, pH 1.0—1.5 for 6 hr, enzyme: substrate=1:100.

The »reference» is denatured (15 min at 40°C) acetic acid-soluble collagen. The »mixture» contains purified $\alpha 2$-, $\alpha 1$-, β_{12}- and β_{11}-components. The designation »control» means that the preparations have not been treated in any way after the purification.

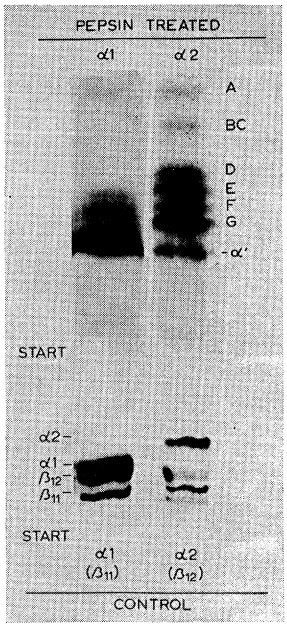

Fig. 2. — Starch-gel electrophoretic patterns of pepsin-treated collagen fractions $\alpha 1+\beta_{11}$ and $\alpha 2+\beta_{12}$ eluted from the CM-cellulose column. The digestion of $\alpha 1+\beta_{11}$ was carried out at 25°C in a HCl-acetic acid buffer, pH 1.0—1.5 for 6 hr, enzyme: substrate=1:100. The digestion of $\alpha 2+\beta_{12}$ was carried out at 25°C in a HCl-acetic acid buffer, pH 1.0—1.5 for 6 hr, enzyme: substrate=1:100.

The »control» samples are studied with electrophoresis as obtained after the fractionation.

(Fig. 1). The $(\alpha 1+\beta_{11})$-fraction was also resistant to pepsin and the patterns resembled those from the $\alpha 1$-component, as expected (Fig. 2). The $\alpha 2$- and $(\alpha 2+\beta_{12})$-fractions were broken down more easily in standard conditions and the mixture yielded the characteristic gel-electrophoretic pattern (Fig. 2).

This observation prompts a recollection of the known differences between the $\alpha 1$- and $\alpha 2$-components. The $\alpha 2$-component is less stable against thermal degradation (2, 4), and it contains less hexose than the $\alpha 1$-component (1). In rat collagen the differences in the contents of aromatic amino acids between the chains are slight but there is an abundance in the $\alpha 2$-component of valine, isoleucine and leucine, but not o alanine (8). However, because the spacing of the polar regions is constant in the various chains (5), we believe that pepsin acts primarily on the analogous linkages and identical patterns of breakdown-products are expected.

We think that the present differences are due to variations, first in the thermal stability of the α-components or their analogous fragments and, second in the amino acid compositions in the sequences where the cleavage occurs. These differences can be utilized for a selective degradation of the components of collagen.

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